HUNTINGTON MEDICAL RESEARCH INSTITUTES
NEURAL ENGINEERING PROGRAM
734 Fairmount Avenue
Pasadena, California 91105

Contract No. N01-NS-1-2340
Quarterly Progress Report
Jan 1-March 31, 2003
Report No.6

"Functional Microstimulation of the Lumbosacral Spinal Cord"

Douglas McCreery, Ph.D.
Victor Pikov, Ph.D
Albert Lossinsky, Ph.D.
Leo Bullara, B.A.
William Agnew, Ph.D.

ABSTRACT

The long-term objective of this study is to develop neural prostheses for spinal cord injured persons unable to voluntarily control their bladder. Our approach is based on implanting an array of microelectrodes into the sacral spinal cord. Microelectrodes can activate small volumes of neural tissue inside the spinal cord that are involved in the micturition reflexes. We employed arrays comprised of either discrete iridium microelectrodes or multi-site silicon substrate probes. The arrays were implanted for one to four months in the S_1-S_2 levels of the spinal cord of adult male cats. We found that charge-balanced stimuli of 00 μA and 400 μsec per phase, delivered at a frequency of 20 Hz at many locations in or near the intermediate zone of the sacral spinal cord, produced micturition-related effects. Depending on their position in the spinal cord, individual microelectrodes have been able to elicit an increase in the hydrostatic pressure within the urinary bladder coincident with either an increase or decrease in the tone of the external urethral sphincter. Microelectrodes that produced a marked activation of the bladder and coincident relaxation of the sphincter were able to induce bladder voiding. A future study will address the question whether spinal cord injured animals can be similarly induced to void by the intraspinal stimulation.

INTRODUCTION

The objective of this project is to develop neuroprostheses that will allow persons with severe spinal cord injuries to regain control of their bladder and bowel. Our approach is to selectively stimulate small volumes of spinal cord tissue involved in micturition reflexes, thereby avoiding the drawbacks associated with stimulation of spinal roots. Penetrating microelectrodes can be designed to target specific areas inside the sacral spinal cord containing bladder-activating and EUS-inhibiting circuitry. In this report, we describe the effect of stimulation at different regions in the S_1-S_2 spinal cord on the bladder and EUS activity.

Several anatomical locations in the sacral spinal cord are specifically involved in the control of the bladder and EUS. A major spinal nucleus, involved in the activation of the bladder is the sacral parasympathetic nucleus (SPN) containing the preganglionic parasympathetic neurons that innervate the bladder detrusor muscle. This nucleus is located at the lateral edge of the intermediate zone at the S_1-S_2 levels in cats and at the S_2-S_3 levels in humans. A major spinal nucleus involved in the relaxation of the EUS is the dorsal gray commissure (DGC), containing the interneurons that inhibit the somatic motoneurons innervating the EUS. This nucleus in the cat is located dorsally to the central canal at the S_1-S_3 levels (Blok et al., 1998).

In the cat, contraction of the bladder can be induced by microstimulating in or near the SPN (Nashold et al., 1971; Carter et al., 1995; Woodford et al., 1996; Grill et al., 1999) and at least a partial relaxation of the EUS can be induced by microstimulating at the DGC (Grill et al., 1999). In the present study, therefore, the chronic intraspinal arrays are designed to target both the SPN and DGC in order to achieve the desired coincident effect on the bladder and EUS. The implantation technique is standardized and is applicable to an eventual translation to clinical practice.

It this study, our microelectrodes penetrated only to a maximum depth of 1.8 mm below the dorsal surface of the cord. This was done in order to minimize the risk of damage to the central canal wall by the microelectrodes, leading to the cerebrospinal fluid leakage. In addition, we wanted to avoid stimulation in the ventral horn, which contains the EUS-projecting and other

somatic motoneurons. Activation of these motoneurons is counterproductive to achieving voiding and can produce unwanted body, tail, or hindlimb movement. Having a goal of eventual clinical application of this neuroprosthesis, we wanted to determine whether activation of voiding reflex is possible at locations in the dorsal half of the cord.

METHODS

Microelectrode array fabrication

The shafts of the discrete iridium microelectrodes are fabricated from iridium wire, 75 μm in diameter. One end of each shaft is etched electrolytically to a cone terminating in a blunt tip with a radius of curvature of 4 to 5 μm. A wire lead is micro-welded near the upper end of the shaft. The shafts and leads wires are insulated with 2-2.5 μm of Parylene-C and the insulation is ablated from the tips of the shafts by an excimer laser operating at 248 nm. The surface area of the exposed tips ranges from 1,600 to 2,400 μm^2.

The individual microelectrodes are assembled into arrays of 9, extending from a superstructure cast from EpoTek 301 epoxy, which is 3 mm in diameter (Figure 1).

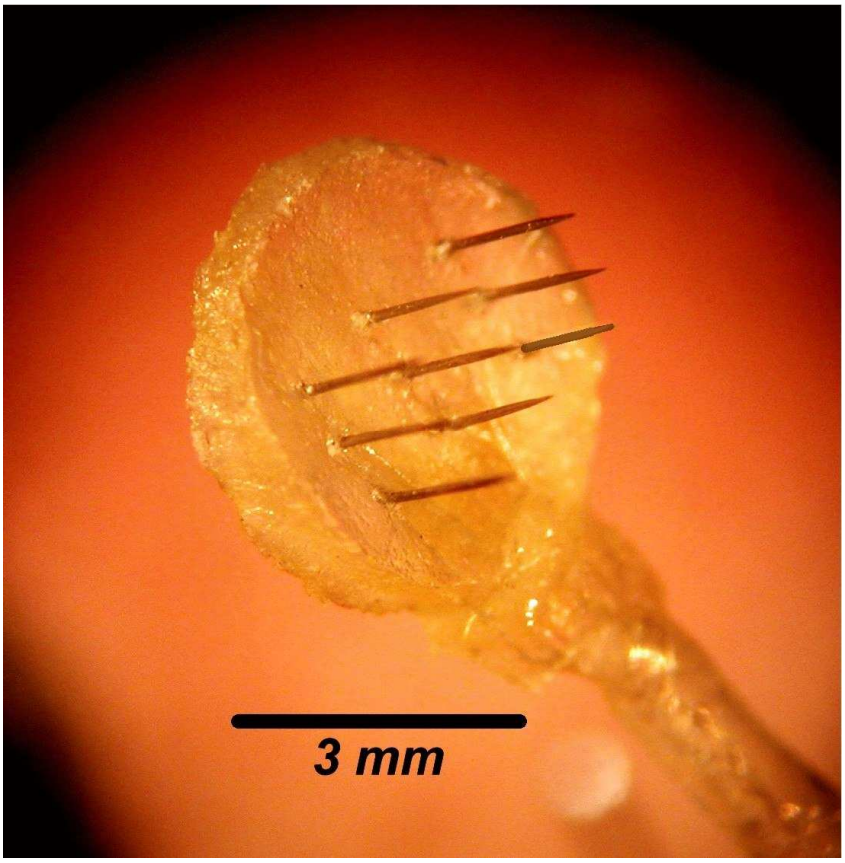


Figure 1. Array of discrete iridium microelectrodes.

The microelectrodes are spaced approximately 500 μm apart in the rostral-caudal dimension, and 0.8 mm in the mediolateral direction. The arrays have incorporated microelectrodes of various lengths, and the 6 electrodes comprising the 2 lateral (outboard) rows

extend 1.4 to 1.7 mm beneath the epoxy superstructure, and the 3 electrodes in the medial row extend 1.3 to 1.6 mm beneath the superstructure. The lateral electrodes are intended to excite the neurons of the SPN, which innervate the bladder detrusor muscle, while the medial electrodes are intended to excite the neurons in and around the central gray commissure, which inhibit the motoneurons innervating the external urethral sphincter.

A second type of array is based on multisite silicon substrate probes. Figure 2 shows a probe that was designed to our specifications by Design Engineer Jamille Hetke of the Center for Integrated Microsystems, at the University of Michigan. Each shank contains 3 electrodes sites that have been sputter-coated with iridium. The sites each have geometric surface areas of approximately 2,000 μm^2 and are located on two of the shanks, at 1.2, 1.5 and 1.8 mm below the probe's transverse spine. The third shank contains no electrodes sites, and is intended to stabilize the implanted array against rotational forces transmitted from the array cable. The probe spines are encapsulated within an epoxy (EpoTek 301) superstructure, to form arrays containing two probes (total of 12 electrodes sites). This array lacks a middle row of electrodes to activate the neurons of the central gray commissure that inhibit the EUS. However, the two probes are spaced only 1.4 mm apart, with the intent that the array will be implanted slightly to one side or the other of the cord's midline and thus one row or the other will be sufficiently close to the midline to excite these neurons.

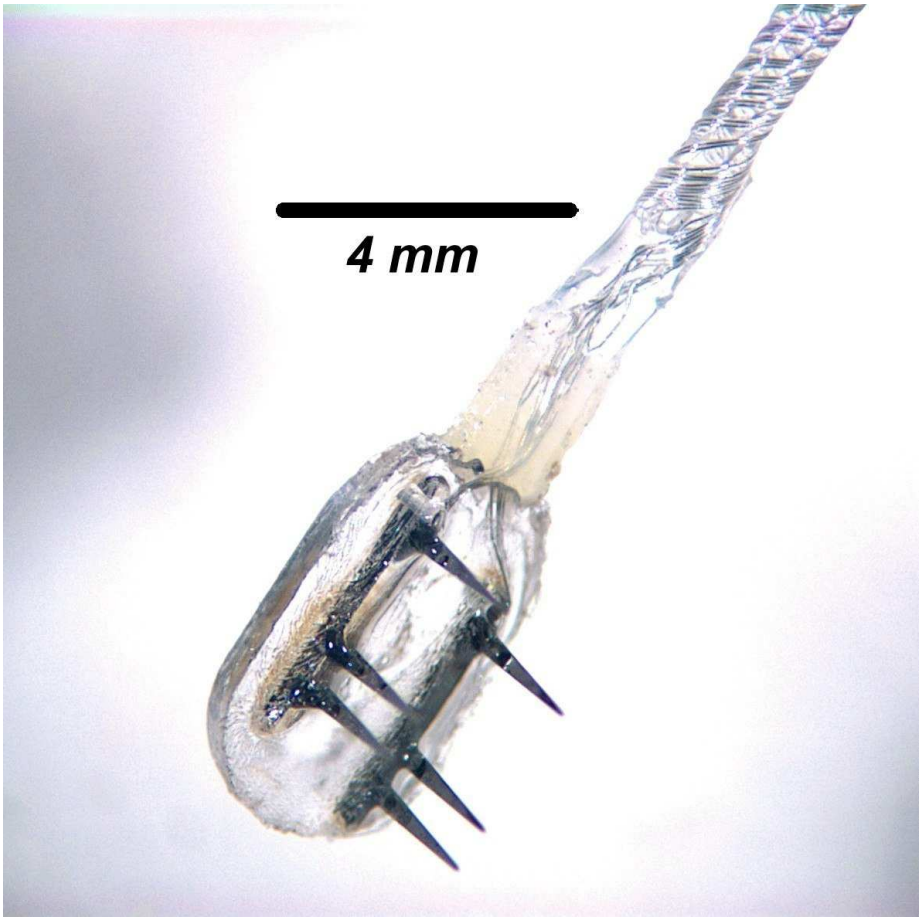


Figure 2. Array of multisite silicon substrate probes.

After assembly, the microelectrodes are "activated" (a layer of high valence iridium oxide is formed by anodic conversion) by potentiodynamic cycling between -0.8 and +0.7 volts with respect to a saturated calomel electrode, while the microelectrodes are immersed in saturated sodium phosphate solution until they have a total charge capacity of approximately 200 nC. The arrays are then cleaned using the "modified Clemson protocol" and sterilized in ethylene oxide in preparation for implantation.

Experimental procedure

Male cats, 1-2 years in age, were used. The arrays were implanted using aseptic technique with animals anesthetized with Halothane and Nitrous oxide. The sacral cord was exposed using a dorsal laminectomy and the dura over the sacral cord was cut longitudinally. To locate the junction of the S_1 and S_2 segments, the perigenital skin was stimulated using a pair of needle electrodes inserted approximately 20 mm apart, while the evoked responses were recorded at several locations along the dorsal surface of the sacral cord. The arrays were implanted near the maximum of the 2^{nd} component of the evoked response (the dorsal cord potential) (McCreery et al., 2003). At autopsy, the position of the array relative to the spinal cord level was validated by a complete dissection of the sacral spinal roots.

The electrode cable and attached microelectrode array was routed from a percutaneous connector mounted on the skull. Each array was aligned relative to the spinal cord surface, placed in contact and inserted using a custom-made inserter tool, at a velocity of approximately 1 m/sec (McCreery et al., 2003). After the array was implanted, a stabilizing pad of polyester mesh, attached to the cable 10 mm from the array superstructure, was tamped onto the dura in order to prevent the transmission of torque and longitudinal forces from the cable to the array. The dura was then closed over the array. For the first two weeks after surgery, animals were housed in a room without perches to reduce spinal mobility and improve array integration with spinal cord tissue.

Following the urodynamic assessment and pulsing of the microelectrodes, the animals were deeply anesthetized and sacrificed by transcardial perfusion. The spinal cord was removed, and transverse sections were cut through the sacral cord and processed for histological and immunohistochemical analyses to identify the location of the microelectrode tips relative to the neuronal groups of interest.

Measurement of bladder pressure and urethral sphincter tone and pulsing of the microelectrodes

We determined the effect of microstimulation in the sacral cord on the hydrostatic pressure within the urinary bladder and on the EUS tone. The cats were anesthetized with Propofol and the urinary bladder was catheterized. The low level of Propofol anesthesia was maintained with the animal breathing on its own and responding to strong sensory stimulation. This allowed monitoring of possible undesired effects of stimulation, such as hindlimb flexion or movement of the tail, or possible painful sensations indicated by generalized body movement and vocalization. If any indications of pain were observed, the stimulation of the microelectrode was immediately discontinued.

Hydrostatic pressure within the bladder vesicle is measured with a transducer (Model 041500503A, Maxxim Medical, Athens, TX), using the apparatus depicted in Figure 3. The bladder was filled with sterile saline until equilibrium was reached with the hydrostatic pressure in the open-top reservoir elevated to a specific height above the bladder. All animals were tested with bladder pressure below the threshold to generate reflex contractions (20-25 mmHg) reflecting a storage phase of the micturition cycle. In addition, some animals were also tested

with high bladder pressure (above 25 mmHg), which induced spontaneous reflex contractions that reflect the voiding phase of the micturition cycle. The constrictive force, or tone, within the EUS was measured with a transducer as an "infusion pressure", the resistance to the infusion of saline through a port on the side of the catheter positioned at the EUS level as saline was infused continuously at a rate of 100 ml/hr. The localization of the EUS was done by slowly moving the catheter along the urethra until the region of maximum infusion pressure was found. The data from the pressure transducers was amplified using a physiological monitor (Model 78534A, Hewlett-Packard, Palo Alto, CA), digitized at a rate of 10 samples per second using a 12-bit data acquisition system (Model PCI-6070E, National Instruments, Austin, TX), and displayed and stored on a computer using a custom program, written in the Visual Basic (Microsoft Corporation, Redmont, WA) using the Measurement Studio ActiveX components (National Instruments, Austin, TX).

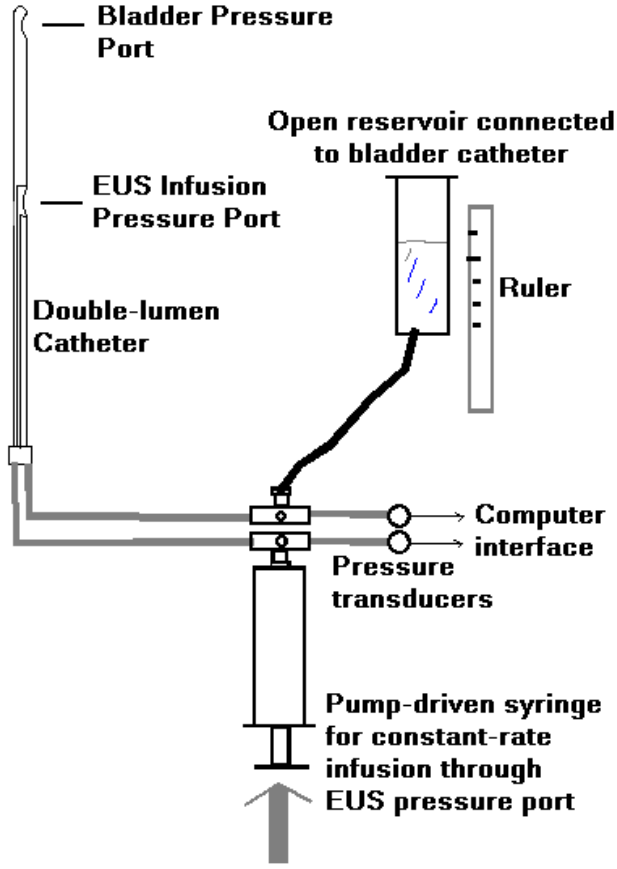


Figure 3. Urodynamic setup.

The train of stimuli was delivered to one microelectrode at a time for 30 sec, followed by a recovery interval of 60 sec before the next train was applied. We used cathodic-first biphasic pulses with duration of 0.4 msec, delivered at a frequency of 20 Hz. This frequency was chosen as optimal for stimulation of bladder and EUS motoneurons based on our previous study (Carter et al., 1995). Changes in bladder pressure and EUS tone were calculated as a difference of average pressures measured during 30 sec of stimulation and during 30 sec immediately before the stimulation (the baseline period).

Histologic Procedures

The cats were deeply anesthetized by i.v. injection of pentobarbital (120 mg/kg), then i.v. heparinized (5000 units) and perfused through the aorta for 30 seconds with a pre-wash solution consisting of phosphate-buffered saline, and 0.05% procaine HCl, followed by 4% formalin fixative, freshly prepared from paraformaldehyde. Tissue blocks containing both the microelectrodes and areas rostral and caudal to the electrodes were post-fixed overnight in 4% formalin, dehydrated and embedded in paraffin. The paraffin-embedded tissue blocks were cut at a thickness of 6-7 μm. The sections were stained with cresyl violet (Nissl stain), and/or for the immunohistochemical marker NeuN. Histologic sections were photographed using a digital microscope camera (Spot RT, Diagnostic Instruments Inc, Sterling Heights, MI)

NeuN immunohistochemistry.

Immunohistochemistry for the neuron-specific protein NeuN, was done using the monoclonal antibody (MAB377, 1:40, Chemicon, Temecula, CA), which selectively binds to proteins within the nucleus (primarily) and cytosol of neurons throughout the nervous system. We employed an antigen retrieval technique in which the sections on glass slides were microwaved for 12 minutes at 70% power in acidic citrate buffer. The immunoreactivity was visualized using biotinylated secondary antibody and peroxidase chromogen (DAB). At the end, the tissue sections were lightly counterstained with cresyl violet.

RESULTS

The present study includes the analysis of 10 cats, which were implanted with the most recent types of the electrode array and were evaluated using the current technique for measuring the bladder pressure and EUS tone. We have implanted the arrays of discrete iridium microelectrodes into the sacral cords of 8 cats, and arrays of multi-site silicon-substrate probes into 2 additional cats. All of the cats displayed normal behavior and no neurological disorders after the implantation until their sacrifice at approximately 2 months after the procedure. The histological appearance of the spinal cords at the implant site is described in detail in our previous report (McCreery et al., 2003). The neurons and glia appeared normal in staining with Nissl and neuron-specific marker NeuN. Within the 50 μm of the electrode tips and shafts, there was variable amount of gliosis with minimal infiltration of inflammatory cells, such as activated macrophages or microglia.

The microelectrode arrays were implanted at either at the S_1 or at the S_1-S_2 junction or at the S_2 level. The responses to intraspinal stimulation at these levels were different and, therefore, have been examined separately. Some of the basic anatomic findings and the microelectrode parameters, based on the implantation level, are summarized in the Table 1. One array of the silicon-substrate probes was implanted at S_1 level, and another – at the S_1-S_2 junction. Each of these arrays had 11 functional electrode sites. The other 8 animals had arrays of the discrete electrodes, a total of 60 of which were functional. All functional electrodes provided sufficient charge capacity for pulsing at 100 μA per phase. Pulsing of two thirds of functional electrodes implanted at the S_1 or S_1-S_2 junction produced an effect on the bladder or EUS, while pulsing of only a third of electrodes implanted at S_2 produced micturition-related responses. This correlated with the results of the dorsal cord potential mapping, where the S_1 and S_1-S_2 junction levels were seen as receiving maximal sensory input from the perigenital area (data not shown). This confirms our justification of using the dorsal cord potential mapping for localizing the sacral spinal cord level(s) that controls the bladder and EUS.

Parameter	Spinal cord level			
	S1	S1-S2 junct.	S2	All levels
Number of animals	4	3	3	10
Animal survival, days	85 (57-120)	73 (30-98)	44 (37-51)	74 (30-120)
Functional els. of both types	35	28	19	82
Discrete els./Silicone substrate els.	24/11	17/11	19/0	60/22
Els. affecting bladder or EUS	24 (69%)	19 (68%)	7 (37%)	50 (61%)
El. access impedance, kOhms (mean ± SD)	63 ± 27	29 ± 9	38 ± 15	50 ± 27
Spinal cord width, mm (mean ± SD)	4.8 ± 0.1	4.3 ± 0.1	3.8 ± 0.1	4.5 ± 0.4

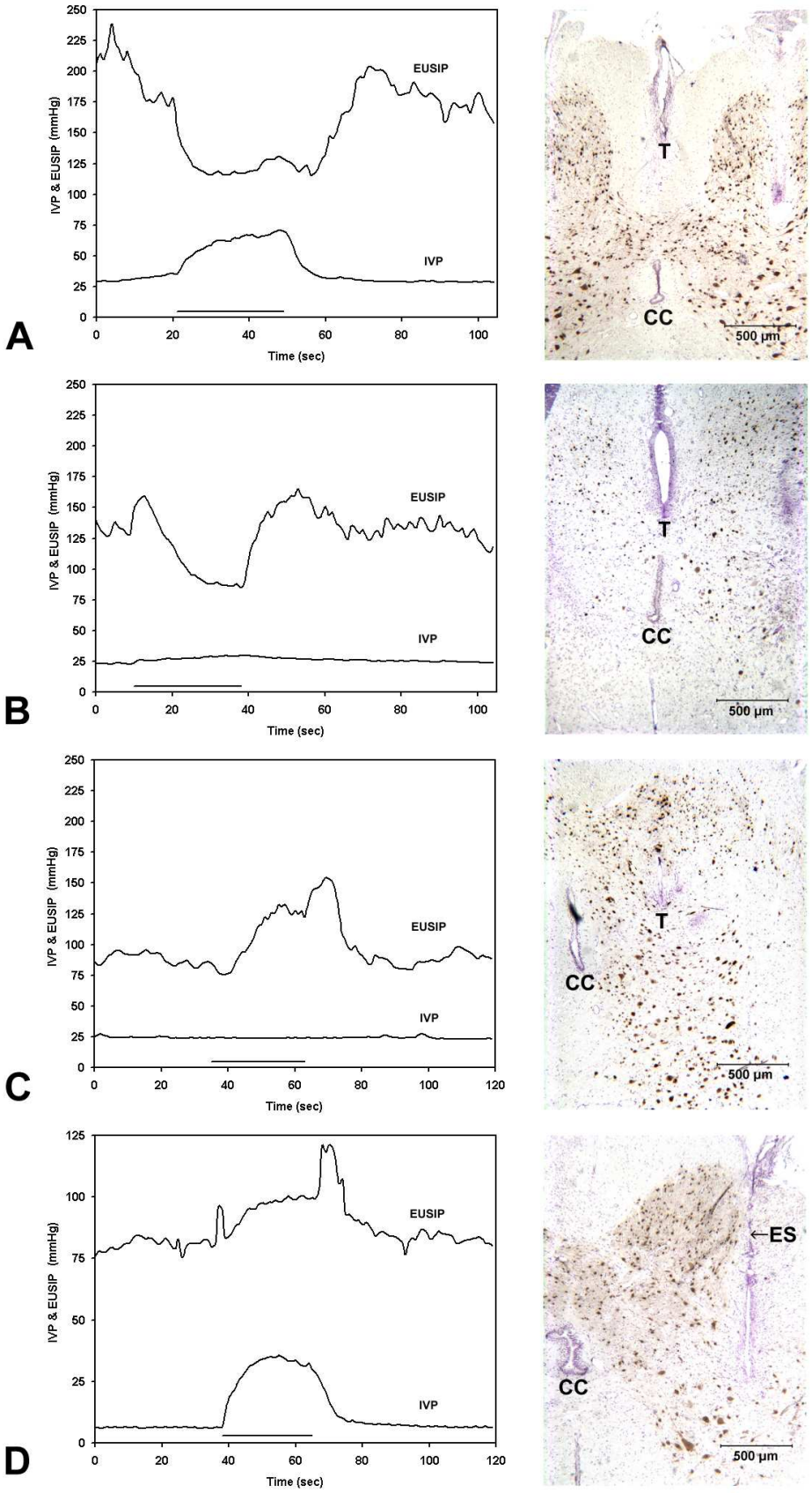
Table 1. Description of the microelectrodes implanted into different sacral cord levels (S_1, S_1-S_2 junction, or S_2). Average animal survival was approximately 2 months, and chronic electrode impedance was about 50 kOhms.

Since the electrodes were targeted to the intermediate zone of gray matter, only a few of them were actually located in the dorsal part of the dorsal horn, containing the pain-processing neuronal circuitry. Thus, we have not observed indications of painful sensations stronger than slight body movement or bilateral hindlimb contraction. The stimulation in these cases was immediately terminated.

Pulsing of individual microelectrodes produced variable effects on the bladder and EUS activity. As presented in a previous report, microelectrodes as close as 500 μm apart produced markedly different effects on the bladder and EUS (McCreery et al., 2003). Figure 4 demonstrates some typical responses. The most desirable effect of microelectrode pulsing is a coincident activation of the bladder and reduction of EUS tone (Figure 4A). There was no evidence of significant bladder or EUS fatigue upon repeated cycles of 30 sec of stimulation and 60 sec without stimulation. This synchronous response was most effective for induction of voiding. In fact, when the catheter was removed from the urethra, pulsing of the microelectrode, represented in Figure 4A, produced a continuous stream of voided urine. In the example shown, the microelectrode tip was located the medial part of the dorsal white matter (the fasciculus gracilis) at the S_1 level. Similar physiological responses were also seen with the tips located at or lateral to the SPN at the S_2 level. This synchronous response was, by far, the most frequently seen micturition-related response in this study.

Pulsing of a few microelectrodes produced a significant inhibition of the EUS tone, but no effect on bladder activity (Figure 4B). In the case shown, the microelectrode tip was located in the area of the DGC immediately above the central canal. Upon repeated stimulation, there was also no significant fatigue in the EUS response. When the catheter was removed from the urethra, pulsing in the dorsal gray commissure produced a weak voiding, which could be supplemented by applying gentle manual pressure to the bladder.

Figure 4 (Next page). Assessment of intravesical pressure (IVP) and EUS infusion pressure (EUSIP) during pulsing of individual microelectrodes. The left panels represent the traces of the IVP and EUS as a function of time, where duration of the applied stimulation is indicated by a solid horizontal line. The right panels are the photomicrographs of the sites of the microelectrodes in the sacral spinal cord. The tips of discrete electrodes (T) or electrode sites of the silicon probes (ES), as well as the central canal (CC) are indicated. The spinal cord tissue was immunostained with NeuN and counterstained with cresyl violet. A through D represent 4 different examples of evoked bladder and EUS activities. See text for further details.


A
B
C
D
IVP & EUSIP (mmHg)
Time (sec)
EUSIP
IVP
T
CC
ES
500 µm

Pulsing of a microelectrode tip located in the intermediate zone at the S1 level produced a marked increase in the tone of the EUS and no significant effect on the bladder (Figure 4C). After withdrawal of the catheter from the urethra, pulsing of this microelectrode produced no voiding. This type of response to pulsing was seen only in 5 microelectrode sites.

Pulsing of microelectrodes located in the dorsal horn sometimes produced indications of painful sensation by the animal, including bilateral hindlimb contraction, and bladder and EUS co-contraction (Figure 4D). In the presented example, the active electrode site of the silicone probe was located on the lateral edge of the middle of the dorsal horn. Since only a few electrodes were located so dorsally and laterally, this response to pulsing was not seen frequently.

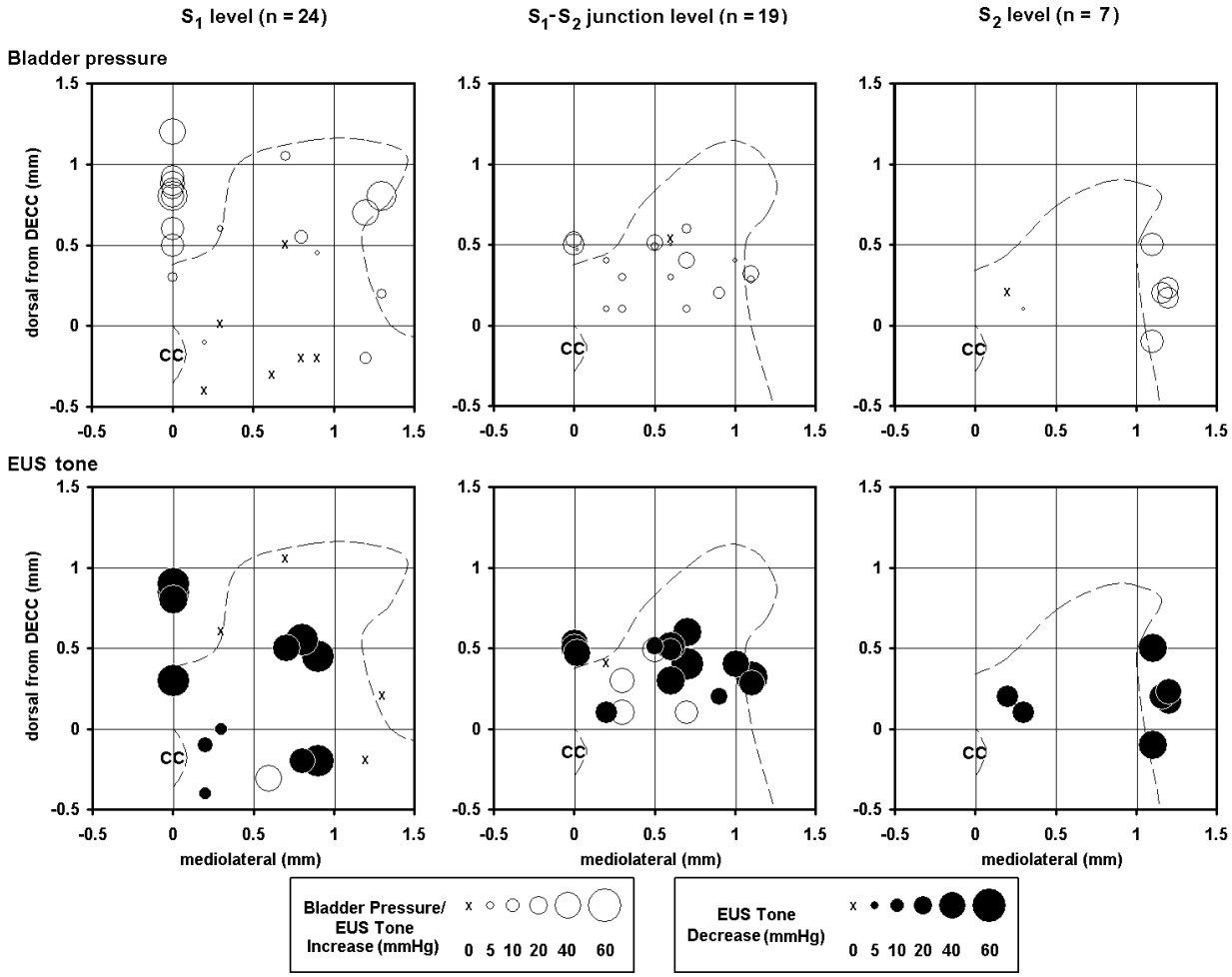


Figure 5. Tip/electrode site locations in the sacral spinal cord of the microelectrodes that produced micturition effects. The circle size indicates the amount of the pressure change from the baseline level. The relationship between the circle sizes and pressures changes is presented below the panels. The circle coordinates indicate the mediolateral and dorsomedial positions in reference to the dorsal edge of the central canal (DECC). The dotted line in each panel represents the gray matter outline and the location of the central canal (CC). The top panels show increases in the bladder pressure (open circles) and the bottom panels show increases (open circles) and decreases in the EUS tone (filled circles) at different microelectrode positions. From left to right, the panels show microelectrode implanted in the S_1, S_1-S_2 junction, or S_2 levels.

Figure 5 summarizes the micturition responses to pulsing of the microelectrodes at the S_1, S_1-S_2 junction, and S_2 levels of spinal cord. The spinal cord outlines are composites of tracings from several animals. The locations of the microelectrodes are marked as circles of different diameter. The diameter of the circles in the top panels corresponds to the amplitude of bladder contractions as measured by intravesical pressure, and the diameter in the bottom panels corresponds to the change in the EUS tone as measured by the infusion pressure, where empty circles represent an increase in the infusion pressure and filled circles represent a decrease.

At the S_1 spinal level, many of the microelectrodes in the medial part of the fasciculus gracilis produced a strong coincident contraction of the bladder and inhibition of the EUS tone. At the S_1-S_2 spinal level, in addition to the white matter just above the DGC, most microelectrodes located in the intermediate zone of the gray matter also produced moderate increases in bladder pressure coincident with the marked inhibition of the EUS tone. At the S_2 level, all microelectrodes located in the white matter just lateral to the SPN, also produced an increase in the bladder pressure and suppression of the EUS tone. Overall, pulsing of most microelectrodes chronically implanted at the intermediate zone and adjacent white matter at the S_1 and S_2 levels evoked a micturition reflex and produced actual voiding upon withdrawal of the transurethral catheter.

In several animals, we have also examined the effect of pulsing on the spontaneously bladder contractions, which began to occur when the baseline bladder pressure exceeded 25 mmHg. In the example shown (Figure 6), pulsing of the microelectrode located in the middle of the dorsal horn produced a marked inhibition of spontaneous bladder contractions and no significant effect on the EUS tone.

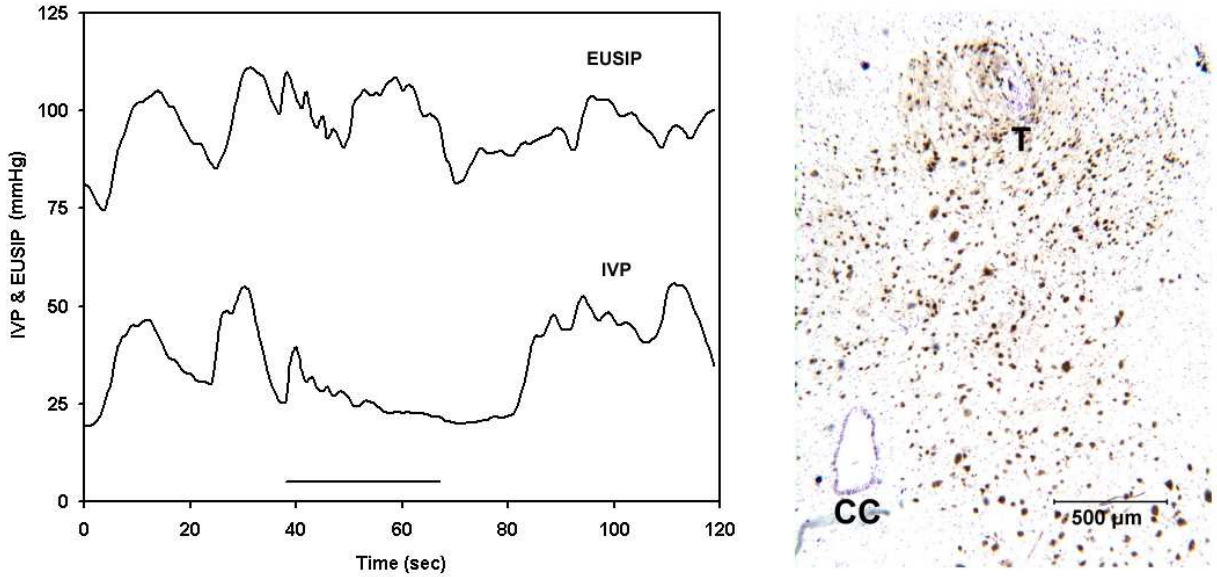


Figure 6. Assessment of intravesical pressure (IVP) and EUS infusion pressure (EUSIP) during pulsing of individual microelectrodes during spontaneous bladder activity. The left panel represents the traces of the IVP and EUS as a function of time, where duration of the applied stimulation is indicated by a solid line. The right trace is the photomicrograph of the microelectrode tip site (T) in the sacral spinal cord. The location of the central canal (CC) is indicated for reference. The spinal cord tissue was immunostained with NeuN and counterstained with cresyl violet.

When the bladder was filled to a pressure below 25 mmHg and there were no spontaneous contractions, the same microelectrode produced a small increase (7 mmHg) of bladder pressure. Similar suppression of the spontaneous bladder activity was seen during pulsing of almost all microelectrodes that produced any significant contraction of the bladder at a low baseline pressure.

DISCUSSION

We have examined the micturition-related responses during pulsing of two types of microelectrode arrays chronically implanted into the sacral spinal cord of the cat. The chronic implantations have permitted measurement of bladder and EUS responses in a lightly anesthetized animal. We found that at the time of testing, all of the functional microelectrodes retained the charge capacity necessary for pulsing at the optimal amplitude of 100 μA. Pulsing of the electrode sites located near the SPN or DGC evoked significant sustained elevation of the bladder pressure of about 40±10 mmHg. Stimulation at these sites in the spinal cord also produced a sustained change in the EUS tone: it was reduced in most cases and increased in a few locations in the gray matter between the SPN and DGC at the S_1-S_2 junction. Several sites located in the dorsal white and gray matter, in addition to evoking bladder contraction, produced flexion of the hindlimbs or movement of the tail. These results concur with and extend the previously published results derived from an acute mapping study in the cat (Grill et al., 1999). That study examined the bladder and EUS pressure (not the EUS tone) using microelectrodes from our laboratory and pulsing parameters similar to these used in our study. However, the stimulus was applied only for 1 sec, and, therefore, the sustainability of the evoked responses was not addressed. A successful intraspinal neuroprosthesis would need to maintain contraction of the bladder and relaxation of the EUS in order to allow a near-complete voiding. In this respect, our study provides a first example of sustained micturition reflex attainable by intraspinal microstimulation.

During the bladder-filling phase of micturition, pulsing of none of the 82 functional electrodes produced a reduction of bladder pressure. Conversely, during the bladder-voiding phase of micturition, pulsing of all of the examined bladder-activating microelectrodes resulted in the inhibition of the spontaneous bladder activity. This suggests a phase-dependent modality of bladder control in the sacral spinal cord. Phase-dependence of the bladder response to the microelectrode pulsing was also reported in the acute cat study (Grill et al., 1999).

There are two major supraspinal descending tracts involved in micturition control. One of these tracts originates in the ventrolateral pontine tegmentum (L-region) and terminates in the Onuf's nucleus and the gray matter area immediately above the SPN at the S_1 level; it is involved in the storage phase of the micturition cycle by keeping the EUS tone elevated (Holstege et al., 1986). Another tract originates in the dorsomedial pontine tegmentum (M-region) and terminates in the intermediate zone at the S_2 level; it is involved in the control of the voiding phase by contracting the bladder (Holstege et al., 1986) and by inhibiting the EUS motoneurons (Blok et al., 1997; Blok et al., 1998). In our study, most of the electrode sites that produced activation of the bladder and suppression of the EUS tone were located in the intermediate zone of the gray matter or lateral to it in the white matter at the S_1-S_2 junction and S_2 level, matching the termination pattern of the M-region fibers. In addition to the intermediate zone, we identified the micturition-inducing electrode sites in the medial part of the fasciculus gracilis at the S_1 level. Previous acute cat study also found that this area produces a strong effect on the EUS (Blok et al., 1998). Yet, this area does not contain fibers from the pontine tegmentum micturtition centers

(Holstege et al., 1986). We propose that induction of micturition from this area is due to activation of the afferent pudendal nerve projections, located in the medial part of the fasciculus gracilis in the caudal L_7 – rostral S_1 in the cat (Ueyama et al., 1984; Thor et al., 1989). These fibers convey sensory information from the urethra and upon stimulation induce a spinal voiding reflex, which is also characterized by coincident contraction of the bladder and inhibition of the EUS (Shefchyk and Buss, 1998).

It was not possible in the present study to determine whether the observed effects on the bladder and EUS were due to stimulation of cell bodies, axons, or dendrites, or some combination of the above. Computer modeling studies indicate that symmetrical cathodic-first pulses used in this study, might preferentially excite large myelinated axons rather than small unmyelinated axons and cell bodies (McIntyre and Grill, 2000; Miller et al., 2001; McIntyre and Grill, 2002). The phase-dependent effect of the stimulation on the bladder accompanied by the inhibition of the EUS suggest that the bladder and EUS motoneurons were activated indirectly via interneurons rather than via direct stimulation of motoneurons.

Sedation by light Propofol anesthesia was required to immobilize the animal to avoid movements of the transurethral catheter. Animals were breathing on their own and responded to strong tactile stimulation. While no other groups have reported the use of Propofol for urodynamic studies in the cat, in the pig Propofol induced no significant inhibition of the parasympathetic bladder contraction reflex, while somewhat suppressing the sympathetic bladder filling reflex (Mills et al., 2000). This resulted in a higher voiding threshold pressure but produced no effect on the maximal voiding pressure and voiding efficiency.

Overall, the results of this animal study indicate the feasibility of achieving effective voiding by pulsing of microelectrode arrays chronically implanted into the sacral spinal cord.

REFERENCES

Blok BF, de Weerd H, Holstege G (1997) The pontine micturition center projects to sacral cord GABA immunoreactive neurons in the cat. Neurosci Lett 233:109-112.

Blok BF, van Maarseveen JT, Holstege G (1998) Electrical stimulation of the sacral dorsal gray commissure evokes relaxation of the external urethral sphincter in the cat. Neurosci Lett 249:68-70.

Carter RR, McCreery DB, Woodford BJ, Bullara LA, Agnew WF (1995) Micturition control by microstimulation of the sacral spinal cord of the cat: Acute studies. IEEE Transactions on Rehabilitation Engineering 3:206-214.

Grill WM, Bhadra N, Wang B (1999) Bladder and urethral pressures evoked by microstimulation of the sacral spinal cord in cats. Brain Res 836:19-30.

Holstege G, Griffiths D, de Wall H, Dalm E (1986) Anatomical and physiological observations on supraspinal control of bladder and urethral sphincter muscles in the cat. J Comp Neurol 250:449-461.

McCreery DB, Lossinsky AS, Pikov V, Bullara LA, Agnew WF (2003) Chronically implantable electrode arrays for functional microstimulation of the lumbosacral spinal cord. Ann Biomed Eng [submitted].

McIntyre CC, Grill WM (2000) Selective microstimulation of central nervous system neurons. Ann Biomed Eng 28:219-233.

McIntyre CC, Grill WM (2002) Extracellular stimulation of central neurons: influence of stimulus waveform and frequency on neuronal output. J Neurophysiol 88:1592-1604.

Miller CA, Robinson BK, Rubinstein JT, Abbas PJ, Runge-Samuelson CL (2001) Auditory nerve responses to monophasic and biphasic electric stimuli. Hear Res 151:79-94.

Mills IW, Drake MJ, Greenland JE, Noble JG, Brading AF (2000) The contribution of cholinergic detrusor excitation in a pig model of bladder hypocompliance. BJU Int 86:538-543.

Nashold BS, Jr., Friedman H, Boyarsky S (1971) Electrical activation of micturition by spinal cord stimulation. J Surg Res 11:144-147.

Shefchyk SJ, Buss RR (1998) Urethral pudendal afferent-evoked bladder and sphincter reflexes in decerebrate and acute spinal cats. Neurosci Lett 244:137-140.

Thor KB, Morgan C, Nadelhaft I, Houston M, De Groat WC (1989) Organization of afferent and efferent pathways in the pudendal nerve of the female cat. J Comp Neurol 288:263-279.

Ueyama T, Mizuno N, Nomura S, Konishi A, Itoh K, Arakawa H (1984) Central distribution of afferent and efferent components of the pudendal nerve in cat. J Comp Neurol 222:38-46.

Woodford BJ, Carter RR, McCreery D, Bullara LA, Agnew WF (1996) Histopathologic and physiologic effects of chronic implantation of microelectrodes in sacral spinal cord of the cat. J Neuropathol Exp Neurol 55:982-991.